Efficient reversal of phiC31 integrase recombination in mammalian cells.

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Public Summary:
More than ten years ago, my laboratory showed that a enzyme found in bacteria that could carry out recombination between chromosomes could also function in human cells. This enzyme, called phiC31 integrase, has became a useful tool for modifying the chromosomes in a wide variety of organisms, including human and mouse cells, as well as cells from fruit flies, plants, toads, and other organisms. For example, we have used phiC31 integrase extensively in gene therapy studies to add healthy genes to mammalian cells that had harmful mutations in various genes, such as the genes involved in hemophilia and muscular dystrophy. The phiC31 integrase enzyme made it possible to add genes, but it was not possible to remove the genes, if desired. In this new study, we show that a newly-identified protein, called excisionase or recombination directionality factor, can reverse the recombination reaction that is carried out by phiC31 integrase. Therefore, it is now possible to carry out the reaction in either direction, to either add a gene or remove a gene. This new feature of the integrase system gives it more utility and flexibility for making changes in chromosomes. In addition, in this study we created a new plasmid, or circle of DNA, that codes for a useful form of phiC31 integrase. The new plasmid, called pCS-kI, encodes a slightly shorter form of integrase. This form can catalyze recombination between the two DNA sequences recognized by the integrase, attB and attP. However, it cannot catalyze recombination between the attB site and sequences in the chromosomes that partially match the attP site. By making the integrase reaction more restrictive, the integrase coded by pCS-kI is more useful when we want the enzyme to react only with the exact attB and attP sites and not other sequences in the chromosomes. In summary, this new study describes new tools that add to the usefulness of the phiC31 integrase system. This recombination system is in wide use by our laboratory and by many other laboratories around the world. Therefore, these new tools will help to stimulate progress in the field of genome engineering, which is an important contributor to the stem cell field.

Scientific Abstract:
Over the past decade, the integrase enzyme from phage phiC31 has proven to be a useful genome engineering tool in a wide variety of species, including mammalian cells. The enzyme efficiently mediates recombination between two distinct sequences, attP and attB, producing recombinant product sites, attL and attR. The reaction proceeds exclusively in a unidirectional manner, because integrase is unable to synapse attL and attR. To date, use of phiC31 integrase has been limited to attP x attB recombination. The factor needed for the reverse reaction--the excisionase or recombination directionality factor (RDF)--was identified recently and shown to function in vitro and in bacterial cells. To determine whether the phiC31 RDF could also function in mammalian cells, we cloned and tested several vectors that permit assessment of phiC31 RDF activity in mammalian environments. In the human and mouse cell lines tested (HeLa, HEK293, and NIH3T3), we observed robust RDF activity, using plasmid and/or genomic assays. This work is the first to demonstrate attL-attR serine integrase activity in mammalian cells and validates phiC31 RDF as a new tool that will enable future studies to take advantage of phiC31 integrase recombination in the forward or reverse direction.